Environmental Health Perspectives
Vol. 54, pp. 163–169, 1984

Biologic Indicators of Cadmium Nephrotoxicity in Persons with Low-Level Cadmium Exposure

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The relationship between abnormal urinary findings and renal function was investigated for 33 Itai-Itai patients, 17 suspected patients, 235 subjects exposed to Cd and 41 subjects not exposed to Cd pollution. High correlation coefficients were observed between urinary findings and renal function in advanced cases of chronic cadmium poisoning, and multiple correlation coefficients between them are as high as 0.73 to 0.86. β_2-microglobulin in urine also correlated closely with renal function and age in slight to moderate cases of cadmium poisoning.

It is concluded that urinary findings such as proteinuria, glucosuria and low molecular weight proteinuria are good indicators of renal dysfunction in moderate to severe cases of chronic cadmium poisoning. In slight to moderate cases β_2-microglobulin in urine has a close relation with renal dysfunction.

Introduction

It is well known that environmental cadmium exposure can cause renal damage. There are many cadmium-polluted areas in Japan, and inhabitants living in some of the cadmium-polluted areas suffer from renal damage (*1*). Renal damage is manifested by abnormal urinary findings, such as low molecular weight proteinuria, glucosuria and aminoaciduria. Renal function tests also reveal both tubular and glomerular dysfunctions (*2,3*). Abnormal urinary findings are usually used as indicators of renal damage caused by environmental cadmium exposure because urine specimens are easy to collect from large numbers of people. It is reported that the prevalence of abnormal urinary findings is significantly higher in cadmium-polluted areas than in nonpolluted areas (*1*).

However, the significance of such urinary findings is not yet completely clear, and there is some controversy regarding the early signs of renal damage. β_2-microglobulin in urine is considered to be one of the early signs of renal tubular damage caused by cadmium exposure. However, β_2-microglobulin level in urine is also said to increase with age and to be independent of renal dysfunction.

The purpose of the present study is to elucidate the significance of urinary findings which are observed in cadmium-polluted areas by comparing urinary findings and renal function tests. In the first part of this paper, the relationships between urinary findings and renal function tests on Itai-Itai patients as well as suspected patients who were considered as the most severe cases of chronic cadmium poisoning are reported. In the second part, results obtained on people living in cadmium-polluted area and showing abnormal urinary findings are examined and compared with values from people living in a nonpolluted area. The effect of age on excretion of β_2-microglobulin in urine was of special interest.

Materials and Methods

In 1976 and 1977, 41 Itai-Itai patients and 17 suspected patients, all female, were admitted to The Kanazawa Medical University Hospital and received a detailed medical examination. Morning urine specimens were collected from them. The following urinary parameters were analyzed by the methods reported earlier: β_2-microglobulin (β_2-mg), retinol binding protein (RBP), lysozyme, glucose, total protein, amino acid, proline, creati-

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Table 1. Biological parameters in Itai-Itai and suspected Itai-Itai patients (N = 50).

	Mean	SE	Range
Urinalysis			
β_2-Microglobulin, mg/g creatinine	191.5	12.6	0–360.5
Retinol binding protein, mg/g creatinine	158.8	11.0	0–325.3
Lysozyme, mg/g creatinine	100.7	11.5	2.3–328.2
Total protein, mg/g creatinine	986	62.6	36–1980
Glucose, mg/g creatinine	3439	420	0–13726
Proline, mg/g creatinine	114.1	14.3	6.4–443.5
Amino nitrogen, mg/g creatinine	617.9	40.7	186.4–1949.0
Cadmium, μg/g creatinine	31.7	7.1	7.0–365.2
Renal function tests			
Creatinine clearance, mL/min	35.7	2.8	3.2–99.5
PAH clearance, mL/min	126.1	12.4	10.6–436
%TRP, %	53.7	2.7	8–86
PSP (15 min), %	11.9	0.9	0–26.5
Bicarbonate, mEq/L	18.5	0.4	10.5–24.1
Age, yr	70.2	1.0	46–82

nine and cadmium (2). It should be noted that β_2-mg and RBP were measured by the single immunodiffusion method in the first part of this study. Renal function was evaluated from determinations of creatinine clearance (C_{cr}), PAH clearance (C_{PAH}), percentage renal phosphorus reabsorption (%TRP), PSP test (15 min) and bicarbonate in arterial blood as measured by ordinary methods. All of urinary examinations and renal function tests could be performed on 33 Itai-Itai and 17 suspected patients.

For the second part of the study, 84 male and 151 female inhabitants in the cadmium-polluted Kakehashi River basin in Ishikawa Prefecture were selected from inhabitants over 50 years of age by performing urine examination for glucose, total protein, amino acid and β_2-mg. The subjects selected showed an abnormal value for at least one of the urinary parameters mentioned above. Morning urine specimens were collected from the selected subjects and analyzed for β_2-mg by radioimmunoassay method (Phadebas β_2-mg test, Pharmacia). C_{cr} and %TRP were used as indicative of renal function. The inhabitants gathered at their community centers early in the morning. Fasting 2-hr urine specimens were obtained after subjects drank approximately 300 mL of water. Blood specimens were drawn midway in the collection period. As controls, 17 male and 24 female inhabitants of a nonpolluted area of Kanazawa city were also examined in the same way.

Results

Urinary Parameters in Itai-Itai Patients

Table 1 presents urinary findings and renal function parameters of 50 subjects examined in The University Hospital. Urinary values were expressed as values per weight creatinine. The ranges of urinary values and renal function parameters were very wide, indicating that conditions of subjects were almost normal to very severe. The mean age was 70 years old. A simple correlation matrix between various biological parameters in urine is shown in Table 2. Most of the parameters, except for cadmium, showed significant correlation. Correlation coefficients between total protein and three kinds of low molecular weight protein were 0.70–0.85. However, signifi-

Table 2. Simple correlation matrix between various biological parameters in urine (N = 50).

	β_2-Micro globulin	RBP	Lysozyme	Glucose	Total protein	Proline	Amino N	Cd
RBP	0.848*							
Lysozyme	0.569*	0.652*						
Glucose	0.413†	0.639*	0.333‡					
Total protein	0.701*	0.846*	0.736*	0.736*				
Proline	0.239	0.458*	0.419†	0.780*	0.621*			
Amino N	0.287‡	0.446*	0.198	0.837*	0.550*	0.704*		
Cadmium	0.168	0.104	−0.008	0.014	0.054	0.109	0.077	
(Age)	(0.238)	(0.256)	(0.339)‡	(−0.163)	(0.097)	(−0.139)	(−0.266)	(0.067)

*Significant, $p < 0.001$.
†Significant, $p < 0.01$.
‡Significant, $p < 0.05$.

Table 3. Simple correlation matrix between different renal function tests (N = 50).

	C_{cr}	C_{PAH}	%TRP	PSP (15 min)	Bicarbonate
C_{PAH}	0.744*				
%TRP	0.540*	0.552*			
PSP (15 min)	0.682*	0.700*	0.517*		
Bicarbonate	0.488*	0.575*	0.571*	0.563*	
(Age)	(−0.272)	(−0.390)†	(0.011)	(−0.388)†	(−0.114)

*Significant, p <0.001.
†Significant, p <0.01.

cant correlations were not observed between cadmium and other parameters.

Table 3 shows a simple correlation matrix between different renal function tests. All of the correlation coefficients were significantly high. Correlation coefficients between urinary parameters and renal function tests are shown in Table 4. All correlation coefficients between low molecular weight protein, total protein and renal function tests were significant (r = −0.39 to −0.67). Glucose showed a significant correlation to %TRP and bicarbonate.

Significant correlations were observed between proline and all renal function tests except C_{PAH}. Amino acid showed a significant correlation only to %TRP. No significant correlation were observed between cadmium and renal function tests. Table 5 shows multiple correlation coefficients between renal function tests and biological parameters in urine. All of the multiple correlation coefficients were significantly high, ranging from 0.73 to 0.84.

Renal Function Parameters for Kakehashi River Basin Inhabitants

Table 6 presents the mean values of C_{cr}, %TRP, β_2-mg and age of subjects from polluted areas and control subjects. The renal functions were significantly lower in subjects from the Cd-polluted areas than in the controls. Correlations between β_2-mg in urine and C_{cr} or %TRP in for both sets of subjects are presented in Figures 1 and 2. β_2-mg is expressed on a logarithmic scale.

In subjects from the polluted area, β_2-mg in urine increased with a decrease of C_{cr} or %TRP. However, control subjects did not show such a tendency. Table 7 indicates correlation coefficients for β_2-mg in urine, C_{cr}, %TRP and age in both sets of subjects. All of the correlation coefficients were significantly high among the male and female subjects from the polluted area. In control subjects, correlations were significant only between C_{cr} and age or %TRP in the female.

Table 8 shows partial correlation coefficients for β_2-mg in urine, C_{cr}, %TRP and age in both sets of subjects. The correlations were significant between β_2-mg and C_{cr} or %TRP when age was fixed in the subjects from the polluted area. Significant correlations were also observed between β_2-mg and age when C_{cr} and %TRP were fixed. However, no significant partial correlations were found in control subjects.

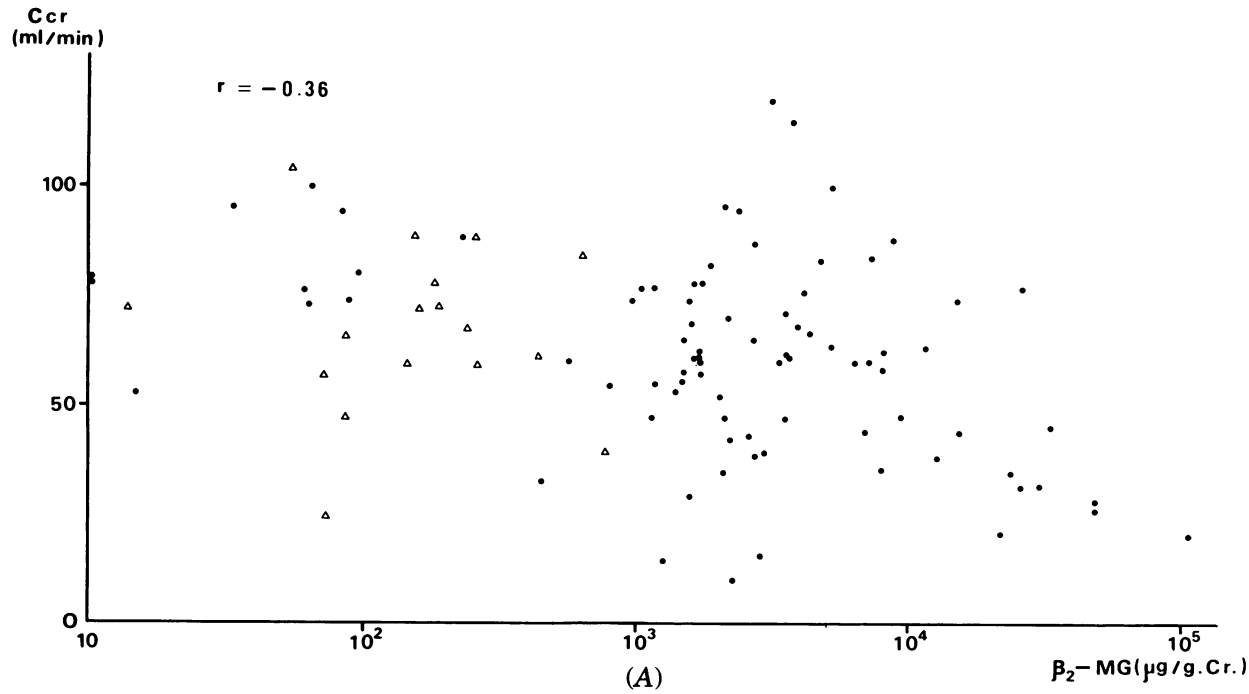
Discussion

Renal damage in residents of the Cd-polluted areas was shown by the existence of abnormal urinary findings and decreased renal function. Abnormal urinary findings included increased excretion of total protein, glucose, amino acids, enzyme and low molecular weight protein such as β_2-mg, RBP or lysozyme. Increased urinary excretion of these substances was more frequently observed in residents of Cd-polluted areas than of nonpolluted areas (*1*). Therefore, these urinary

Table 4. Correlation between biological parameters in urine and renal function tests (N = 50).

	C_{cr}	C_{PAH}	%TRP	PSP (15 min)	Bicarbonate
β_2-Microglobulin	−0.582*	−0.627*	−0.622*	−0.564*	−0.467*
RBP	−0.550*	−0.626*	−0.668*	−0.625*	−0.515*
Lysozyme	−0.387†	−0.546*	−0.599*	−0.566*	−0.593*
Total protein	−0.404†	−0.595*	−0.669*	−0.507*	−0.618*
Glucose	−0.274	−0.274	−0.613*	−0.274	−0.468*
Proline	−0.294‡	−0.254	−0.628*	−0.366†	−0.483*
Amino N	−0.027	−0.052	−0.460*	−0.036	−0.196
Cadmium	−0.117	−0.079	−0.275	−0.015	0.083

*Significant, p <0.001.
†Significant, p <0.01.
‡Significant, p <0.05.



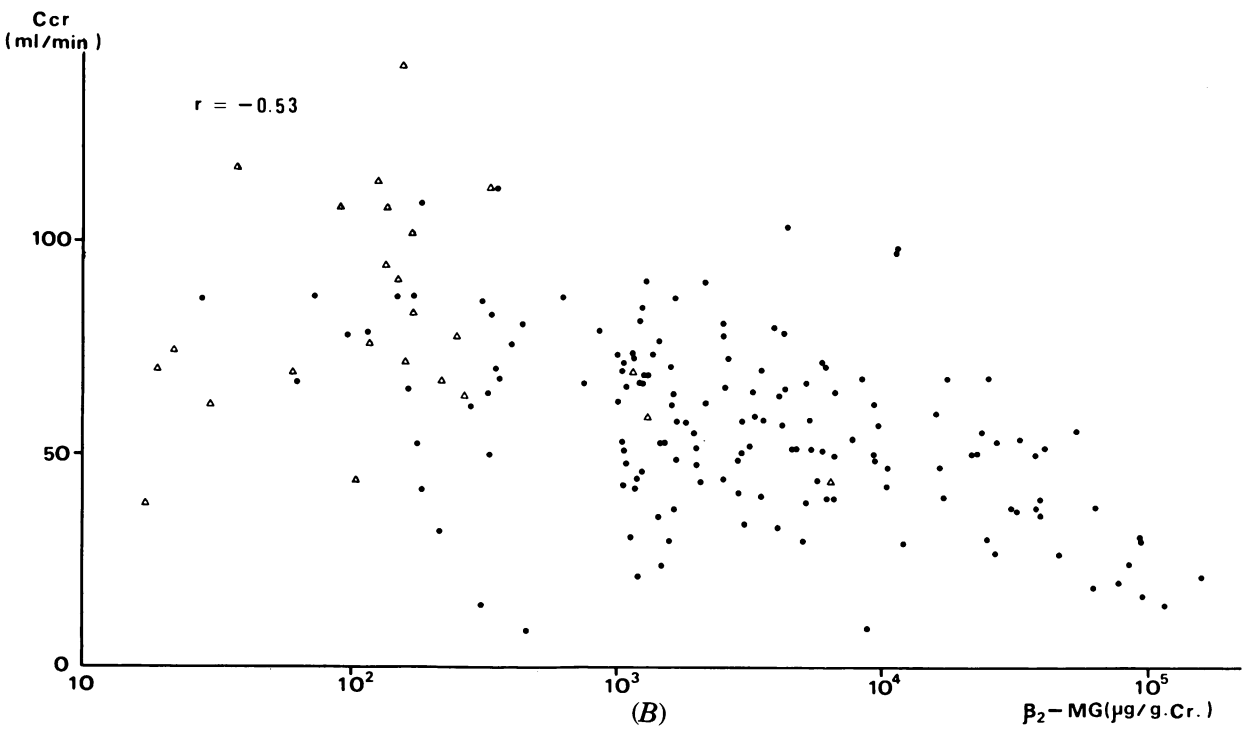


FIGURE 1. Correlation between creatinine clearance and β_2-microglobulin in urine of (*A*) males and (*B*) females: (Δ) controls; (●) residents of Cd-polluted area.

Table 5. Multiple correlation coefficients between renal function tests and biological parameters in urine.

	C_{cr}	C_{PAH}	%TRP	PSP (15 min)	Bicarbonate
Biological parameters in urine	0.732*	0.735*	0.841*	0.782*	0.760*

*Significant, $p < 0.001$.

Table 6. Biological parameters in subjects from Cd-polluted area and controls.

	Males				Females			
	Polluted area (N = 84)		Controls (N = 17)		Polluted area (N = 151)		Controls (N = 24)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
C_{cr}, mL/min	61.2	2.5	67.1	4.7	56.4	1.7	81.6	5.3
%TRP, %	73.5	1.2	88.8	1.5	78.0	0.9	89.0	1.2
β_2-mg, μg/g creatinine	2004.5[a]	1.2	153.8	1.3	3013.0[a]	1.2	144.5	1.3
Age, yr	69.5	1.1	65.4	1.5	68.5	0.7	64.4	2.2

[a]Geometric mean.

findings are considered to indicate the existence of renal damage caused by environmental cadmium exposure. However, the clinical significance of the abnormal urinary findings remains a question, specifically in relation to clinical renal tubular dysfunction.

Increased excretion of β_2-mg in urine at an early stage of cadmium exposure is considered to be a more sensitive parameter than other abnormal urinary findings. However, some reports indicate a close association between increase of β_2-mg in urine and age (*4,5*). Therefore, an increase of β_2-mg in urine at an early stage of cadmium exposure is not considered significant in relation to renal functions (*6*). Tsuchiya et al. (*7*) reported that an increase of β_2-mg at an early stage of exposure is caused by a mechanism other than renal tubular dysfunction, most likely an increase of β_2-mg in blood.

The first part of this study was performed to make clear the clinical significance of the abnormal urinary findings observed in residents of Cd-polluted areas by comparing them with renal function tests. The target group was Itai-Itai patients and patients who were suspected to suffer from advanced cases of chronic cadmium poisoning. The renal function tests revealed that they had moderate to severe glomerular and tubular dysfunction. There was a significantly high correlation among urinary findings except cadmium.

Renal function tests also showed a significant high correlation with each other, indicating that in advanced cases of chronic cadmium poisoning the urinary parameters and renal functions parallel each other. It should be noted that age had no close association with urinary or renal function parameters. The high correlation coefficients be-

Table 7. Simple correlation matrix between various biological parameters in subjects from polluted areas and controls.[a]

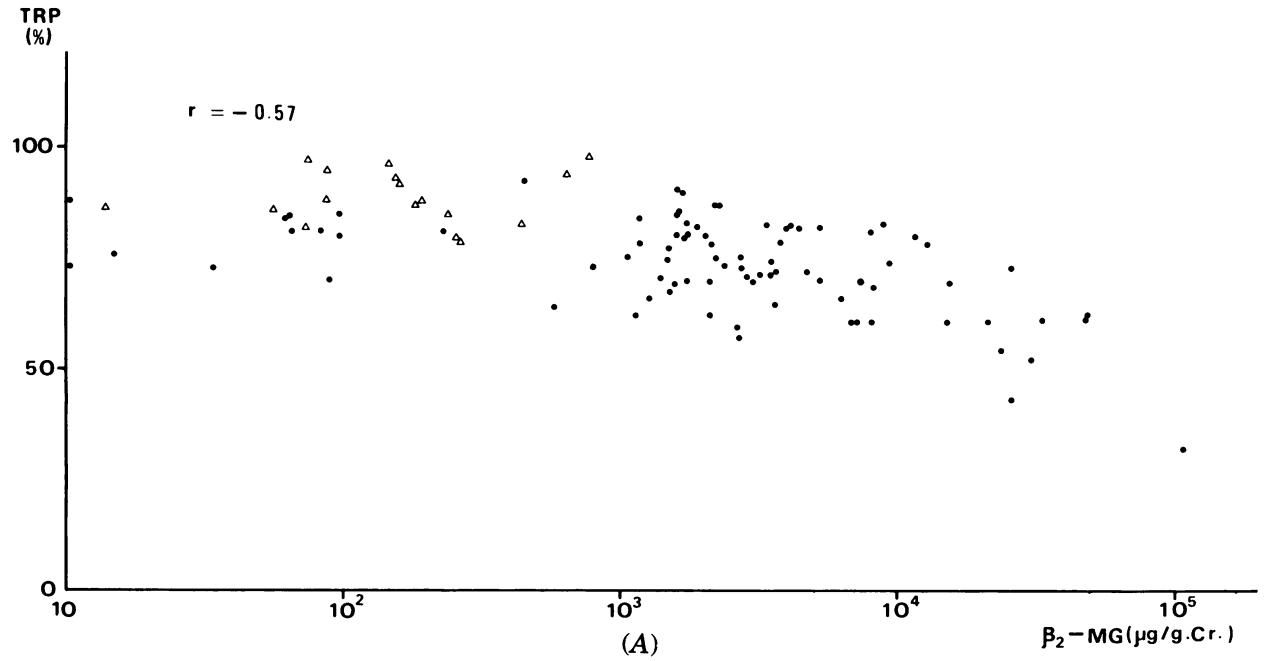
Sex	Parameter	β_2-Microglobulin	C_{cr}	%TRP
Males	C_{cr}	−0.384*		
		(−0.048)		
	%TRP	−0.472*	0.381*	
		(0.107)	(−0.323)	
	Age	0.503*	−0.593*	−0.312†
		(0.420)	(−0.207)	(0.018)
Females	C_{cr}	−0.473*		
		(−0.105)		
	%TRP	−0.561*	0.428*	
		(0.060)	(−0.466)‡	
	Age	0.502*	−0.485*	−0.230†
		(0.220)	(−0.519)‡	(0.155)

[a]Correlations for controls in parentheses.
*Significant, $p < 0.001$.
†Significant, $p < 0.01$.
‡Significant, $p < 0.05$.

Table 8. Partial correlation coefficients for β_2-microglobulin with C_{cr}, %TRP and age in subjects from Cd-polluted area and controls.

	Males		Females	
Correlation	(N = 84) Polluted area	(N = 17) Control	(N = 151) Polluted area	(N = 24) Control
β_2-mg—C_{cr} • Age	−0.123	0.044	−0.303*	0.011
β_2-mg—%TRP • Age	−0.384*	0.110	−0.529*	0.027
β_2-mg—Age • C_{cr}, %TRP	0.356†	0.428	0.383*	0.154

*Significant, $p < 0.001$.
†Significant, $p < 0.01$.



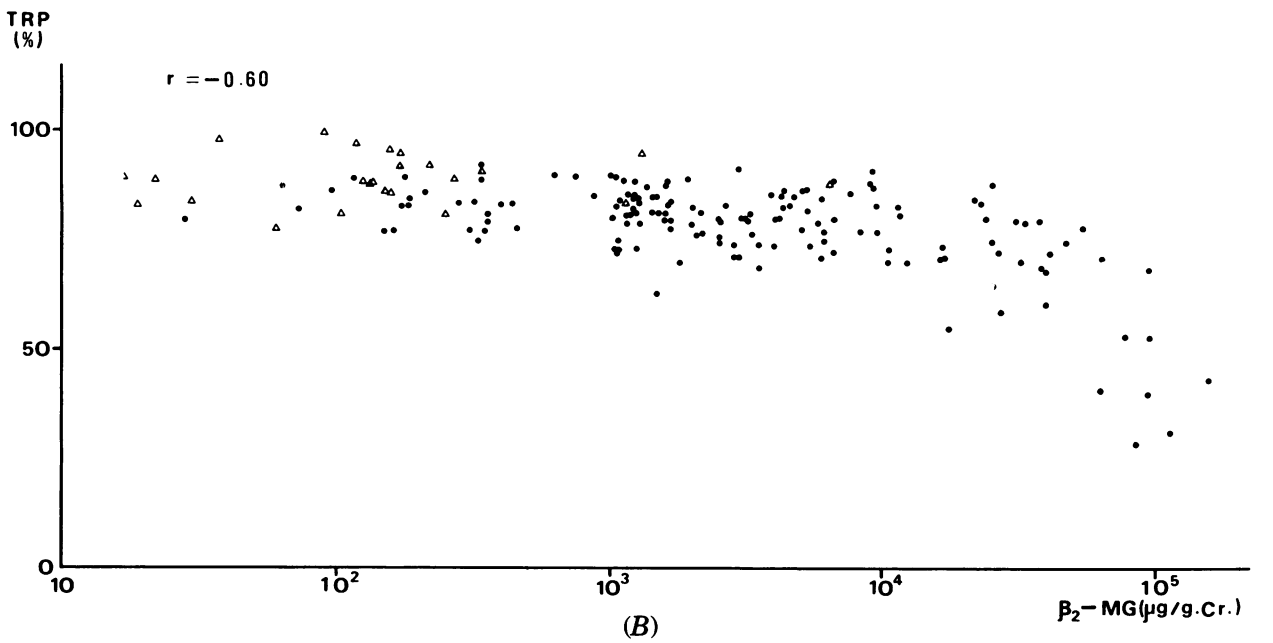


FIGURE 2. Correlation between %TRP and β_2-microglobulin in urine of (*A*) males and (*B*) females: (Δ) controls; (●) residents of Cd-polluted area.

tween urinary findings and renal function demonstrated that urinary findings have a close relationship with renal function. The multiple correlation coefficients between renal function tests and urinary findings were as high as 0.73 to 0.84. This means that renal functions could be estimated from the urinary parameters.

In the second part of this study, the significance of early urinary findings of chronic cadmium poisoning were examined. β_2-mg was used as an early urinary sign, and the target group was the inhabitants of a Cd-polluted area who showed at least one positive urinary finding, such as proteinuria, glucosuria or β_2-microglobulinuria.

Renal function of the residents of the Cd-polluted area was better than that of Itai-Itai patients; however, a significant decrease of renal function was observed compared with control subjects. Therefore the renal functions of the residents of the Cd-polluted area were better than those of Itai-Itai patients and poorer than those of the control subjects.

Correlations between β_2-mg and C^{cr}, %TRP or age were significant in the residents of the Cd-polluted area. Partial correlations for these subjects were also significant between β_2-mg and renal function or between β_2-mg and age when age or renal function was fixed. The control subjects did not show such significant partial correlations. It is reasonable to say from these facts that β_2-mg in urine has a significant relation to renal dysfunction and age in the early stage of chronic cadmium poisoning. However, the influence of age on β_2-mg in urine of polluted subjects must be much less than that of renal dysfunction. In general, age has two aspects, namely, as a physiological factor and as a measure length of exposure to cadmium. It is usually difficult to separate these two to clarify the influence of age on urinary excretion of β_2-mg. We believe that the influence of physiological factors was much less than that of duration of long-term exposure to cadmium for β_2-mg in urine. In the early stages of cadmium exposure both factors may have an effect on the excretion of β_2-mg into urine.

In conclusion, the present study indicates that urinary findings are good indicators of renal function in advanced cases of chronic cadmium poisoning. β_2-mg in urine has also significant meaning in evaluating renal function in the early stage of chronic cadmium poisoning.

The author thanks Prof. S. Shinoda for providing the data on renal functions of Itai-Itai and suspected patients.

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